INTERVENOR'S EXHIBIT 203

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Veterinary Parasitology 125 (2004) 397–407
veterinary parasitology
www.elsevier.com/locate/vetpar

Efficacy of fipronil/(S)-methoprene combination spot-on for dogs against shed eggs, emerging and existing adult cat fleas (Ctenocephalides felis, Bouché)

D.R. Young[a], P.C. Jeannin[b,c], A. Boeckh[b,c,*]

[a] Young Veterinary Research Services, Turlock, CA, USA
[b] Merial, Saint-Vulbas, France
[c] Duluth, GA, USA

Received 23 March 2004; received in revised form 13 July 2004; accepted 25 July 2004

Abstract

The inhibitory activities of fipronil (10% (w/v) solution), (S)-methoprene (9% (w/v) solution), and fipronil/(S)-methoprene (10 and 9% (w/v) solution, respectively) combination against eggs and emerging adult cat fleas (Ctenocephalides felis) and adulticidal activity were tested on experimentally infested dogs. Thirty-two Beagle dogs were selected for this study and eight replicates of four animals were formed based on body weight within sex. One dog in each replicate was randomly allocated to treatment with: (1) untreated control; (2) fipronil 10% (w/v) solution, (3) (S)-methoprene 9% (w/v) solution, and (4) fipronil 10% (w/v) and (S)-methoprene 9% (w/v) combination solution. Treatments were applied once topically on Day 0 at the rate of 0.067 ml/kg. On Days −12, −1, 21, and weekly to Day 84 each dog was infested with ~200 fleas and comb counted ~24 h later, or 2 days (our 48 h) after in the case of Day −1 infestation. On Days −11, 1, 22, and weekly to Day 85 each dog was again infested with ~200 fleas. Flea eggs were collected over ~24 h beginning 3 days after infestation. Fleas were combed off of the dogs and counted at the end of the egg collection period (~96 h count). One aliquot of up to about 100 eggs, if available, from each animal at each infestation time was incubated for ~72 h to determine larval hatch and the other for 35 days to determine the number of adults that developed.

The 10% (w/v) fipronil spot-on provided excellent control (>95%) of adult fleas on dogs for 5 weeks. Similarly, the combination spot-on of 10% (w/v) fipronil and 9% (w/v) (S)-methoprene

* Corresponding author. Tel.: +1 678 638 3634; fax: +1 678 638 3636.
E-mail address: albert.boeckh@merial.com (A. Boeckh).

doi:10.1016/j.vetpar.2004.07.021

provided excellent control of adult fleas, i.e., >95% for 5 weeks. From week 6 post-treatment onward, the relatively low inhibition of adult flea emergence substantiated the lack of significant ovicidal/larvicidal activity in the fipronil (10%, w/v) treatment group. However, the combination product provided excellent (>90%) ovicidal activity for 8 weeks and high (91.4%) inhibition of adult flea emergence for 12 weeks. In addition, a synergistic effect of the two compounds in combination was demonstrated with fipronil enhancing the ovicidal and inhibition of adult flea emergence activity of (S)-methoprene against cat flea eggs.

When all stages of the life cycle of the cat flea are considered, the combination spot-on product provided a high level of total flea control yielding a curative effect against adult fleas and inhibition of flea development stages with little to no potential reinfestation pressure on the animal or in the environment for 12 weeks.

Keywords: Fipronil; (S)-Methoprene; Ctenocephalides felis; Control method-Arthropoda

1. Introduction

Up to the mid-1900s, flea control was achieved through repeated application of on-animal products and premise application of insecticides and insect growth regulators (IGRs) (Marchiondo, 1993; Dryden and Prestwood, 1993). Most insecticides effectively eliminated existing adult fleas from the host during the initial treatment, but reinfestation was a common occurrence. Compliance by pet owners to consistently follow on-animal and premise treatment programs was most difficult and insecticide treatment lacked adulticidal activity beyond a couple of weeks, pets repeatedly acquired new fleas from the premises and infestations remained a recurring if not a continuous problem.

The recent development of insecticides and IGRs in convenient spot-on dosage forms with prolonged residual activity has dramatically improved pet owner compliance and helped to eliminate recurrent infestations.

2. Materials and methods

This study complied with the principles of both the FDA/CVM guidance entitled Good Target Animal Study Practices: Clinical Investigators and Monitors and the European Union Good Clinical Practice for the Conduct of Clinical Trials for Veterinary Medicinal Products.

2.1. Animals

Thirty-two (24 male and 8 female) healthy Beagle dogs between 12.7 and 65.9 months old and weighing 10.2–19.8 kg on Day −3 were selected for the study from a total of 38 dogs. Dogs were washed with water and a mild shampoo and combed once on Day −14. Six dogs with the lowest pre-allocation adult flea counts (from Day −11) were dropped from the trial. The dogs were identified by a unique tattoo in the ear.

2.2. Test substances

The test substances were: 10% (w/v) fipronil, 9% (w/v) (S)-methoprene, and 10% (w/v) fipronil plus 9% (w/v) (S)-methoprene in solutions using the vehicle for a proprietary spot-on formulation. The control dogs were untreated.

2.3. Experimental design

The study followed a randomized block design where dogs were ranked by descending Day −3 body weight into gender-based groups. Within each group, the dogs were assigned to replicates of four dogs each. Dogs were randomly allocated to one of the following four treatment groups by random number generator (Statview for Macintosh, version 4.5, Abacus Concepts Inc.): Group 1, untreated control; Group 2, 10% (w/v) fipronil administered at the minimum recommended dose volume of 0.067 ml/kg (i.e., 6.7 mg/kg); Group 3, 9% (w/v) (S)-methoprene administered at the minimum recommended dose volume of 0.067 ml/kg (i.e., 6.0 mg/kg); and Group 4, 10% (w/v) fipronil and 9% (w/v) (S)-methoprene combination administered at the minimum recommended dose volume of 0.067 ml/kg (i.e., 6.7 mg/kg fipronil and 6.0 mg/kg (S)-methoprene).

Dogs were weighed on Day −3 for dosage calculations. On Day 0, the appropriate test article was applied to dogs in treatment groups 2–4. The hair coat was parted between the shoulder blades on the dorsal midline, to expose the skin. A tuberculin syringe was used to apply the calculated volume of test material directly to the skin. In order to prevent run-off and loss of material, the dog was restrained until the test material had spread evenly over the skin. The study was conducted such that all personnel involved in parasite infestation, counting and evaluation of results were blinded to the treatment.

2.4. Management

Animals were managed similarly and with due regard for their well-being. Dogs were individually housed in plastic crates with wire mesh floors for egg collections. During the time periods between egg collections, the dogs were individually housed in 4 × 10 ft runs with concrete floors and solid metal 4 ft barriers between each run.

Standard handling precautions were taken to prevent contamination between groups. Dogs in the untreated control group were handled on a separate bench or table than that used for treated groups and combed with different flea combs. Separate plastic crates for egg collection were used for each treatment group.

2.5. Parasitological techniques

Approximately 200 unfed, adult cat fleas (*Ctenocephalides felis*), obtained from an established closed colony maintained on cats, were applied to each animal along the dorsal midline on Days −12, −11, −1, 1, 21, 22, 28, 29, 35, 36, 42, 43, 49, 50, 56, 57, 63, 64, 70, 71, 77, 78, 84 and 85. Care was taken to avoid placement of fleas directly to areas of treatment application, by application to the caudal lumbo-sacral area.

Live fleas were removed from each animal via combing and counted at ~24 h post-infestation or post-treatment, on Days −11, −7, 1, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85. Additional adulticidal data were obtained from the fleas used to provide the eggs for the ovicidal evaluations (counted ~96 h after each infestation) on Days 5, 26, 33, 40, 47, 54, 61, 68, 75, 82 and 89.

On Days −8, 4, 25, 32, 39, 46, 53, 60, 67, 74, 81, and 88, food and water were restricted in an effort to prevent soiling of the egg collection. The clean, dry waste pans were lined with a paper substrate that facilitates the collection of flea eggs. The shed flea eggs were collected overnight. From each animal's egg collection, two aliquots of ~100 flea eggs each were placed into four Petri dishes, with ~25 eggs per dish. If less than 200 eggs was available, the entire production was divided equally into two aliquots with the number of eggs in each dish recorded. Aliquot 1 was used to test efficacy of treatment for inhibition of egg eclosion, whereas flea growth medium was added to the Petri dishes of Aliquot 2 to test the efficacy of treatment for inhibition of adult flea emergence. All Petri dishes were incubated at ~24–30 °C and 70–85% RH. Petri dishes of Treatment Group 1 were held in a separate incubator from that used for Treatment Groups 2–4 to prevent contamination of the untreated control group.

Thirty-five days after each egg collection, Petri dishes from Aliquot 2 were agitated by vigorous shaking, exposed to CO_2 and then frozen prior to counting the number of emerged adult fleas. Pupae that had not released adult fleas were dissected, the encased adult examined, assessed for normal development, and counted. Fully developed encased adults were included with the number of emerged adult fleas for data analysis. Seventy-two hours after each egg collection, Petri dishes from Aliquot 1 were observed, and larval hatch were counted and recorded.

2.6. Statistical analysis

Flea counts were transformed to ln(count + 1) for analysis and calculation of geometric means at each time point. The number of larvae that hatched was divided by the number of Aliquot 1 eggs incubated and the number of adults that developed (live emerged plus fully developed, encased adults) was divided by the number of Aliquot 2 eggs incubated. These proportions for each animal at each time point were transformed to radians using the arcsine square root transformation and transformed counts and proportions were analyzed at each time point using analysis of variance for a completely randomized design. At some post-treatment time points, treatment groups were compared using a t-test for unequal variances because of variance heterogeneity among the groups. Also, the interaction of the effect of fipronil and the effect of (S)-methoprene was tested. A significance level of 0.05 was used.

3. Results

High and consistent percentages of egg hatch, adult flea emergence and adult flea burdens throughout the study duration provided means to accurately assess mean efficacy (Tables 1–3). The fipronil/(S)-methoprene combination product as compared to untreated controls had significantly ($P < 0.01$) lower proportions of larvae that hatched and adults that

Table 1
Summary of percentage[a] of larvae (*C. felis*) that hatched for controls and treated dogs with percent efficacy for dogs treated topically with fipronil, (S)-methoprene, or the combination on Day 0

Infestation day[b]	Untreated control	Fipronil (10%, w/v)	(S)-Methoprene (9%, w/v)	Fipronil (10%, w/v)/ (S)-methoprene (9%, w/v)
Pretreatment	74.9	71.9	71.0	68.4
Day 1	77.6	–[c]	0	–
%Efficacy		–[c]	100	–
Day 22	76.4	–	0.1	–
%Efficacy		–	99.8	–
Day 29	75.0	–	0.9	–
%Efficacy		–	98.9	–
Day 36	63.7	–[d]	2.7	–
%Efficacy		–[d]	95.8	–
Day 43	78.8	51.6[e]	5.3	1.8[f,g,h]
%Efficacy		34.5[e]	93.9	97.8[f]
Day 50	75.1	49.4[i]	6.0	6.8[g,h,j]
%Efficacy		34.3	92.0	90.9[j]
Day 57	76.8	56.2[i]	29.7	1.4[g,h,k]
%Efficacy		26.8	61.3	98.2
Day 64	78.0	59.2	30.0	8.5[g,h,k]
%Efficacy		24.2	61.5	89.1
Day 71	75.3	62.7	30.6	8.1[g,h,k]
%Efficacy		16.7	59.3	89.3
Day 78	85.3	49.4	30.6	12.4[g,h,l]
%Efficacy		42.1	64.1	85.5
Day 85	76.1	61.8	50.4	26.9[g,h,k]
%Efficacy		18.8	33.7	64.6

[a] Retransformed mean of radians; based on the transformation arcsine $\sqrt{(\text{number of larvae/number of eggs})}$.
[b] Eggs were collected for ~24 h starting ~72 h after infestation. Approximately 100 eggs were incubated for ~72 h.
[c] One animal with one egg incubated, no larvae hatched.
[d] One animal with 36 eggs incubated; 8 larvae hatched.
[e] Six animals with eggs incubated.
[f] Three animals with eggs incubated.
[g] $P < 0.01$ vs. untreated control.
[h] $P < 0.05$ vs. fipronil (10%, w/v).
[i] $P < 0.05$, fipronil × (S)-methoprene interaction.
[j] Seven animals with eggs incubated.
[k] $P < 0.05$ vs. (S)-methoprene (9%, w/v).
[l] $P = 0.059$ vs. (S)-methoprene (9%, w/v).

Table 2
Summary of percentage[a] of adults cat fleas (C. felis) that developed and percent efficacy for controls and dogs treated topically with fipronil, (S)-methoprene, or the combination on Day 0

Infestation day[b]	Untreated control	Fipronil (10%, w/v)	(S)-Methoprene (9%, w/v)	Fipronil (10%, w/v)/ (S)-methoprene (9%, w/v)
Pretreatment	55.3	58.0	57.5	56.1
Day 1	56.5	–[c]	0	–
%Efficacy		–[c]	100	–
Day 22	57.4	–	0.1	–
%Efficacy		–	99.8	–
Day 29	55.5	–	0.8	–
%Efficacy		–	98.5	–
Day 36	56.9	–[d]	0.5	–
%Efficacy		–[d]	99.2	–
Day 43	59.2	20.5[e]	2.5	0.7[f,g,h]
%Efficacy		65.4[e]	95.8	98.8[f]
Day 50	53.5	23.2	4.7	2.2[g,h,i]
%Efficacy		56.7	91.3	95.9[i]
Day 57	57.4	37.3	13.8	0.3[g,h,j]
%Efficacy		35.0	75.9	99.4
Day 64	55.5	39.2	18.9	3.8[g,h,j]
%Efficacy		29.5	66.1	93.1
Day 71	53.5	45.7	13.9	2.2[g,h,j]
%Efficacy		14.6	74.0	95.9
Day 78	59.0	27.0	16.0	3.6[g,h,j]
%Efficacy		54.1	72.9	93.9
Day 85	52.6	38.9	19.1	4.5[g,h,j]
%Efficacy		26.0	63.7	91.4

[a] Retransformed mean of radians; based on the transformation arcsine $\sqrt{(\text{number of adults/number of eggs})}$.
[b] Eggs were collected for ~24 h starting ~72 h after infestation. Approximately 100 eggs were incubated for ~35 days.
[c] One animal with one egg incubated, no adults developed.
[d] One animal with 35 eggs incubated; 4 adults developed.
[e] Six animals with eggs incubated.
[f] Three animals with eggs incubated.
[g] $P < 0.01$ vs. untreated control.
[h] $P < 0.05$ vs. fipronil (10%, w/v).
[i] Seven animals with eggs incubated.
[j] $P < 0.05$ vs. (S)-methoprene (9%, w/v).

developed for each of the infestations from Days 43 through 85 (Tables 1 and 2). The combination product had significantly ($P < 0.05$) lower proportions of larvae and adult fleas than the group treated with fipronil alone for Days 43 through 85 infestations (Tables 1 and 2). Likewise, the combination product had significantly ($P < 0.05$) lower proportions of larvae

and adult fleas than the group treated with (S)-methoprene alone for Days 57–85 infestations (except Day 78 infestation for larvae, $P = 0.059$). Based on larval hatch (Table 1), excellent control (>95% efficacy) was achieved with the fipronil/(S)-methoprene combination product and (S)-methoprene alone for 6 and 5 weeks, respectively. Excellent control of adult flea development was achieved with the combination product and (S)-methoprene alone for at least 8 and 6 weeks, respectively (Table 2).

Dogs treated with the fipronil/(S)-methoprene combination product had significantly ($P < 0.05$) fewer adult fleas than controls at each post-treatment counting day through Day 82 (except Day 57, $P = 0.055$, Table 3). Compared to dogs treated with (S)-methoprene alone,

Table 3
Summary of geometric mean[a] flea (*C. felis*) counts 24 h after infestation and percent efficacy for controls and dogs treated topically with fipronil, (S)-methoprene, or the combination on Day 0

Infestation day	Counting day	Untreated control	Fipronil (10%, w/v)	(S)-Methoprene (9%, w/v)	Fipronil (10%, w/v)/ (S)-methoprene (9%, w/v)
Pretreatment	Day −11	163.0	159.9	171.9	166.1
Day −1	Day 1[b]	139.0	9.0	85.5	2.6[c,d]
%Efficacy			93.5	38.5	98.1
Day 21	Day 22	122.6	0.1	115.7	0.2[c,d]
%Efficacy			99.9	5.6	99.8
Day 28	Day 29	144.0	0.6	119.3	0.1[c,d]
%Efficacy			99.6	17.2	99.9
Day 35	Day 36	161.7	0.7	122.4	0.3[c,d]
%Efficacy			99.6	24.3	99.8
Day 42	Day 43	140.3	7.7	131.8	22.5[c,d]
%Efficacy			94.5	6.0	83.9
Day 49	Day 50	127.8	27.0	107.9	46.1[c,d]
%Efficacy			78.9	15.6	63.9
Day 56	Day 57	127.5	67.2	112.5	64.4[e]
%Efficacy			47.3	11.7	49.5
Day 63	Day 64	121.3	70.6	106.0	60.1[c,d]
%Efficacy			41.8	12.6	50.5
Day 70	Day 71	124.7	96.5	119.3	88.7[c]
%Efficacy			22.6	4.3	28.9
Day 77	Day 78	107.6	71.1	95.0	62.1[c,d]
%Efficacy			33.9	11.7	42.3
Day 84	Day 85	151.9	132.2	121.7	93.2[c]
%Efficacy			13.0	19.9	38.7

[a] Retransformed mean; based on the transformation ln(count + 1).
[b] Count was conducted ~24 h after treatment, or 48 h after infestation.
[c] $P < 0.05$ vs. untreated controls.
[d] $P < 0.05$ vs. (S)-methoprene (9%, w/v).
[e] $P = 0.055$ vs. untreated controls.

those treated with the combination product had significantly ($P < 0.05$) fewer fleas through Day 50, and periodically after that.

The interaction of fipronil effect and (S)-methoprene effect was tested to determine if any consistent pattern was present. The interaction was significant ($P < 0.05$) for larval hatch (Days 50 and 57 infestations, Table 1) and count of adult fleas (96 h count on Days 5, 26, 33, and 82, Table 4). The interaction was not significant ($P > 0.05$) for adult development or flea counts at 24 h after infestation (Tables 2 and 3, respectively). The interaction in 96 h counts (Table 4) through Day 33 was apparently related to the fact that both groups receiving fipronil (alone or in combination) had no fleas (100% efficacy) and the (S)-methoprene group had a low level of efficacy (30–40%).

The 10% (w/v) fipronil spot-on product provided excellent control (>95%) of adult fleas on dogs for 5 weeks (Table 3). Similarly, the combination spot-on product of 10% (w/v)

Table 4
Summary of geometric mean[a] flea (*C. felis*) counts and percent efficacy 96 h after infestation for controls and dogs treated topically with fipronil, (S)-methoprene, or the combination on Day 0[a]

Infestation day	Counting day	Untreated control	Fipronil (10%, w/v)	(S)-Methoprene (9%, w/v)	Fipronil (10%, w/v)/ (S)-methoprene (9%, w/v)
Pretreatment	Day −7	146.8	124.9	137.2	128.7
Day 1	Day 5	128.8	0[b]	89.4	0
%Efficacy			100	30.6	100
Day 22	Day 26	89.6	0[b]	51.6	0
%Efficacy			100	42.3	100
Day 29	Day 33	138.9	0[b]	87.8	0
%Efficacy			100	36.8	100
Day 36	Day 40	135.4	0.8	73.4	0
%Efficacy			99.4	45.8	100
Day 43	Day 47	127.5	7.9	78.5	7.6
%Efficacy			93.8	38.5	94.0
Day 50	Day 54	121.0	27.1	82.3	23.7
%Efficacy			77.6	32.0	80.4
Day 57	Day 61	131.2	32.2	107.8	19.8
%Efficacy			75.4	17.8	84.9
Day 64	Day 68	126.7	98.4	99.1	90.2
%Efficacy			22.3	21.7	28.8
Day 71	Day 75	119.7	93.1	97.4	68.6
%Efficacy			22.2	18.6	42.7
Day 78	Day 82	127.0	83.2[b]	67.4	74.4
%Efficacy			34.5	47.0	41.5
Day 85	Day 89	104.8	119.6	87.9	65.9
%Efficacy			0	16.1	37.1

[a] Retransformed mean; based on the transformation ln(count + 1).
[b] $P < 0.05$, fipronil × (S)-methoprene interaction.

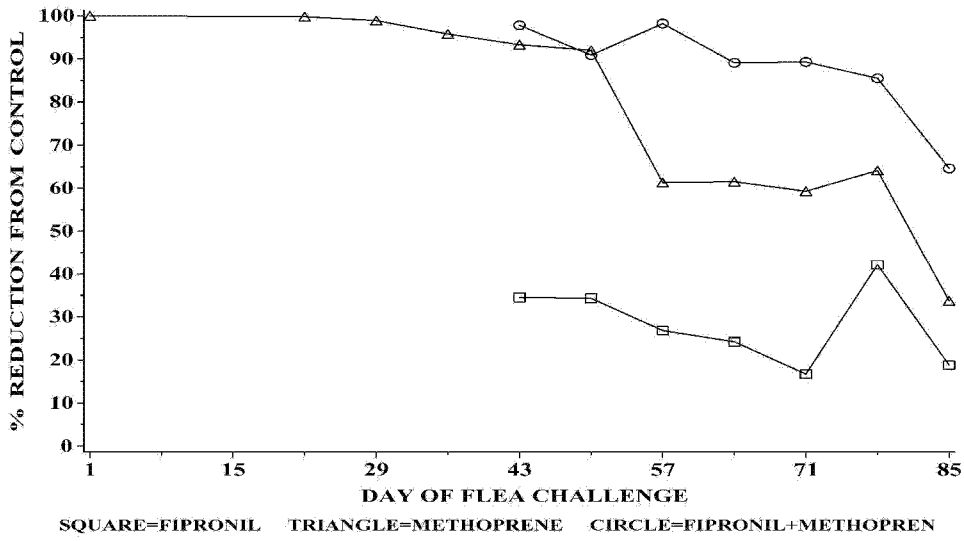

Fig. 1. Percent reduction in proportion of larvae (C. felis) that hatched by treatment and day of flea challenge (eight dogs per treatment group).

fipronil and 9% (w/v) (S)-methoprene provided excellent control of adult fleas, i.e., >95% for 5 weeks. From week 6 post-treatment onward, the relatively low inhibition of adult flea emergence substantiated the lack of any significant ovicidal/larvicidal activity in the 10% (w/v) fipronil treatment group. Conversely, the combination product provided excellent (>90%) ovicidal activity for 8 weeks and high (91.4%) inhibition of adult flea emergence for 12 weeks (Tables 1 and 2, respectively). A larvicidal effect was observed between the larval hatch and lack of adult flea emergence on Days 64, 71, 78 and 85 (Table 2). No treatment related health problems were observed with any of the treatments.

Figs. 1 and 2 provide graphic presentations of tabular data in Tables 1 and 2, respectively. These graphs show that the percent reduction in larval hatch and adult emergence of the fipronil/(S)-methoprene combination is significantly enhanced over the percent reduction in larval hatch and adult flea emergence of the (S)-methoprene treatment alone. Fig. 1 shows the percent reduction in larval hatch of the combination to be enhanced (~1.5 times) over (S)-methoprene alone from Days 57 to 85. Likewise, the percent reduction of adult development of fipronil/(S)-methoprene treatment was enhanced by ~1.3 times over (S)-methoprene alone (Fig. 2). The inhibitory activities of fipronil/(S)-methoprene combination against the flea egg and larval development to the emerged adult flea is greater than (S)-methoprene alone.

4. Discussion

The known modes of action of fipronil and (S)-methoprene against insects are unrelated and distinct. Fipronil is an extremely active insecticide/acaricide belonging to the

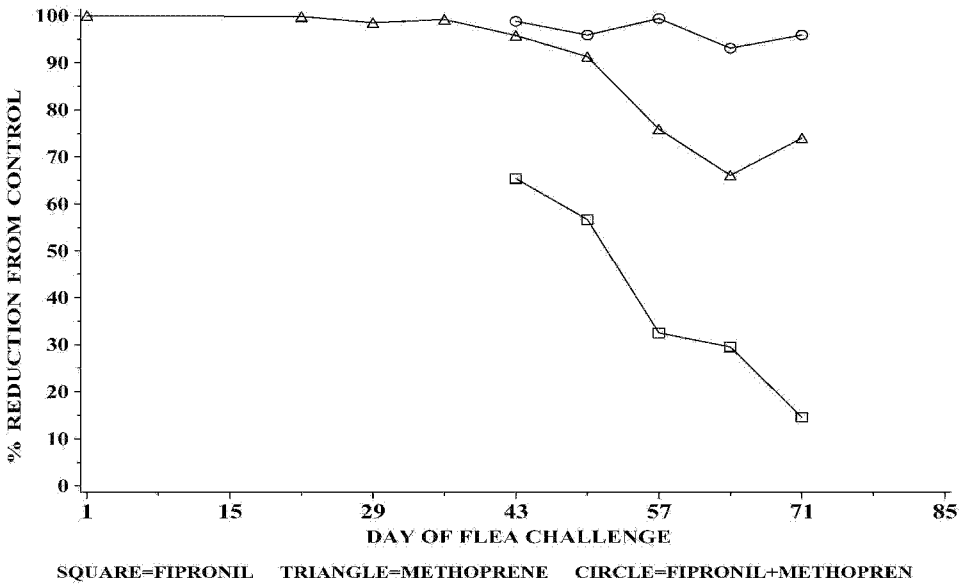

Fig. 2. Percent reduction in proportion of adult fleas (C. felis) that developed by treatment and day of flea challenge (eight dogs per treatment group).

phenylpyrazole family interacting with ligand-gated chloride channels, specifically those gated by the inhibitory neurotransmitter γ-aminobutyric acid (GABA), thus blocking pre- and post-synaptic transfer of chloride ions through the integral transmembrane chloride channel of the $GABA_A$ receptor (Cole et al., 1993).

(S)-Methoprene is a synthetic juvenile insect hormone analog (JHA) that causes impaired development and mortality of the developing stages of the cat flea (Moser et al., 1992). (S)-Methoprene on the hair provides high and persistent activity against immature stages of the cat flea by interfering with the development of eggs and subsequent inhibition of adult flea emergence (Olsen, 1985; Donahue and Young, 1992). (S)-Methoprene has no lethal activity against adult fleas and is often formulated with a fast-acting and/or residual insecticide to kill adult fleas on the host.

Because the mechanism of action for each chemical is independent from each other, no pharmacological interferences would be expected and no enhancement of the ovicidal activity of (S)-methoprene in the presence of fipronil would be anticipated. However, data from this report demonstrated an apparent synergistic effect of the two compounds in combination where fipronil enhanced the ovicidal and inhibition of adult flea emergence activity of (S)-methoprene against cat flea eggs. The ability of GABA antagonists, like fipronil, to specifically target $GABA_A$ receptors may make the developing nervous system of insects especially vulnerable to neurotoxins. Any activity of fipronil on the developing insect embryonic tissue within the flea egg coupled with the action of (S)-methoprene on the prevention of blastokinesis (Riddiford and Williams, 1967; Palma et al., 1993) may provide a synergistic effect. Thus, inhibition of neuronal and somatic cellular development during flea egg oogenesis, as well as interference with the structural integrity of the chorion

(Meola et al., 1996; Marchiondo et al., 1999), are potential ovicidal activities of fipronil and (S)-methoprene in combination.

In conclusion, when all activities of the combination spot-on product are considered against the life cycle stages of the cat flea, the fipronil/(S)-methoprene combination spot-on product provided a high level of total flea control yielding a curative effect against adult fleas on the dogs and inhibition of flea developmental stages with little to no potential reinfestation pressure on the animal or in the environment for 12 weeks. Specifically, the combination spot-on provided >95% adulticidal activity for 5 weeks, >90% ovicidal activity for 8 weeks, and >90% inhibition of adult flea emergence for 12 weeks. With the addition of (S)-methoprene to fipronil, the resulting combination spot-on now provides a single product to (1) break the cat flea life cycle under field conditions in spite of the lack of compliance to regular/monthly applications, (2) reinforce the efficacy of fipronil against adult fleas, and (3) provide long-term stewardship of the fipronil molecule since the combination of two different modes of action is will delay the development of flea resistant strains to fipronil.

References

Cole, L.M., Nicholson, R.A., Casida, J.E., 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pest. Biochem. Physiol. 46, 47–54.

Donahue, W.A., Young, R., 1992. Evaluating a synergized pyrethrin/(S)-methoprene spray against feline flea infestations. Vet. Med. 87, 999–1007.

Dryden, M.W., Prestwood, A.K., 1993. Successful flea control. Comp. Cont. Educ. Pract. Vet. 15 (6), 821–830.

Marchiondo, A.A., 1993. Safe and effective flea control for cats. Vet. Technol. 14 (4), 235–243.

Marchiondo, A.A., Meola, S.M., Palma, K.G., Slusser, J.H., Meola, R.W., 1999. Chorion formation and ultrastructure of the egg of the cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 36 (2), 148–157.

Meola, R.W., Pullen, S., Meola, S., 1996. Toxicity and histopathology of the growth regulator pyriproxyfen to adults and eggs of the cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 33, 670–679.

Moser, B.A., Koehler, P.G., Patterson, R.S., 1992. Effects of methoprene and diflubenzuron on larval development of the cat flea (Siphonaptera: Pulicidae). J. Econ. Entomol. 85, 112–116.

Olsen, A., 1985. Ovicidal effect on the cat flea, Ctenocephalides felis (Bouché), of treating fur of cats and dogs with methoprene. Int. Pest Contr. 27 (1), 10–13.

Palma, K.G., Meola, S.M., Meola, R.W., 1993. Mode of action of pyriproxyfen and methoprene on eggs of Ctenocephalides felis (Siphonaptera: Pulicidae). J. Med. Entomol. 30 (2), 421–426.

Riddiford, L.M., Williams, C.M., 1967. The effects of juvenile hormone analogues on the embryonic development of silkworms. Proc. Natl. Acad. Sci. U.S.A. 57, 595–601.